Colette PAUPARDIN*†, Claude LEDDET** & Roger GAUTHERET***:

Histo-chemical investigations on fennel. Physiological connection between terpen's nature and histological structure of secretory apparatus (1)

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ウイキョウの組織化学的研究. テルペン類と分泌器官の組織学的・生理学的関連 (1)

Fennel is an aromatic plant belonging to the family Umbelliferae, elaborating an essential oil containing a rather high concentration of anethol and a smaller amount of three other phenylpropanes and seven monoterpenes.

Preliminary analysis revealed that the essential oil extracted from the various organs of the plant exhibits differences in regard to the quantitative and even the qualitative compositions; these differences affect specially phenylpropanes.

The more drastic difference concerns anethol which exists practically only in the aerial organs whereas dillapiol is notably present only in the roots. This characteristic besides being very sharp suggests that the secretory apparatus of fennel does not consist of long and anastomosed canals but of short and independent units. This must be specially true for the collar which is the limit between stem and root: at this level, a very important variation of essential oil composition can be observed. These facts encouraged us to compare on one hand the essential oil compositions of the different organs and on the other hand the histological structure of the secretory apparatus.

Before describing our own results, it is necessary to summarize the data published previously. Curiously, some works concerned essential oil composition and neglected the histological point of view while others, inversely, threw the histological facts into relief. For these reasons, the two kinds of results will be presented separately.

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History As early as 1687, Malpighi, who initiated histology had discovered secretory canals; 180 years later, secretory canals were observed in fennel by Trécul (1866). He thought that the secretory canals extended to the whole plant; between 1784 and 1884, the secretory canals of Umbelliferae and specially of fennel were described by Colignon (1874), Planchon (1875), Moynier de Villepoix (1878), Van Tieghem (1872-1885), and Haberlandt (1914); but really detailed investigations were performed only since 1950. Metcalfe & Chalk (1950) claimed that the secretory canals have the same structure in all organs; this statement was disproved by Bruch (1956) who reported that the root contains two kinds of canals. Cerceau-Larrival (1962) mentioned the presence of secretory canals in hypocotyl.

Canals disposition of the fruits was carefully studied by Gupta (1964) and Shah et al. (1969). They reported that the fennel's fruit contains six large secretory canals and sometimes small supernumerary.

The observations of Gupta (1964) and those of Shah et al. (1969) were corroborated by Kadry et al. (1978); they indicated, in addition, that stem, leaf and petals contain canals located in front of vascular bundles. Kadry et al. (1978) described the presence of a kind of nectary at the top of the ovary; they indicated the presence of canals in the pith of stem and inflorescential stalk; the leaf lamina contain a single canal. Canals of the stem's cortex are 34 to 77 μm in diameter while those of the pith are only 17 to 32 μm; on a transversal section the ovary exhibits twelve canals.

Analysis of the fennel essential oil began at the end of the last century. First, Tardy (1897) analysed the essential oil of a bitter fennel coming from the south of France. He detected the presence of monoterpenic hydrocarbons such as pinene, cymene and phellandrene and, in addition, fenchone (called fenone) estragol, anethol, anisaldehyde and an intermediary compound: $C_{13}H_{14}O_2$. Perrot (1944), in his exhaustive book on plant natural products, verified the histological structure of fennel's fruit and reported that there were no connections between its canals and the secretory apparatus of the peduncle. Estimations indicated that the fruit contains 2 to 6% of essential oil; this has 60-70% of anethol and 10-20% of fenchone; moreover Perrot indicated the presence of other products in low concentrations: estragol and some terpenic hydrocarbons. Japanese and Indian fennels have fenchone and french fennel does not. Several factors such as ripening of fruit influences essential oil contents; Gleisberg &

Hartrott (1960) have shown that during the ripening, the oil concentration increased and after that, reduced until the full ripening.

Toth (1967) considered the composition of essential oil extracted from fennels growing in different countries; Germany, Holland, Hungary etc.…, in regard to fruit morphology and habit of the plant. Gas and thinlayer chromatographic analysis established particularities of sweet fennel (low containt of pinene and fenchone, and high concentration of limonene), and of bitter fennel (high concentration of fenchone, pinene, low containt of limonene).

Toth (1967) does not only point out the qualitative differences between fruit and leaf essences; he indicated for the first time the presence of a high concentration of dillapiol (90%) in essential oil extracted from roots. In opposition with Hoppe (1958), Toth detected neither fenchone nor anethol or estragol in the root. He investigated the action of climatic variations in one place (Karlsruhe) on the content and composition of essential oil; he recorded only weak variations and concluded that this composition is genetically controlled.

Chingova (1967), working on both sweet and bitter fennels, reported that for a single variety, the amount of essential oil of the fruit differs according to the place of harvesting; moreover sweet fennel has less essential oil than the bitter variety, but its oil has a higher concentration in anethol. Similar analysis performed by Karlsen et al. (1969) confirmed this result and showed, in addition, that bitter fennel contains more pinene and myrcene than the sweet variety, but on the contrary is less rich in limonene. This is in agreement with Toth's result.

According to Tsvetkov (1970), the central number of sweet fennel contains more anethol than the peripheric ones, whereas it is the contrary with the bitter fennel. Trenkle (1969-1972) compared two bitter fennels harvested in RDA and Rumania; he indicated for the first time a slight presence of monoterpenic hydrocarbons in the roots like myristicine and parsley apiol associated with anethol and dillapiol. He remarked that stems and leaves contain relatively more monoterpenics hydrocarbons than fruits and that it is the opposite for fenchone and anethol. Ashraf & Bhatty (1975) assigned the same commercial value to fennels coming from France, Pakistan and Russia. Masada (1976) and later Kraus & Hammerschmidt (1980) realised analysis of fennel essential oil. Merkes (1980), comparing different fennels, reported like Toth that sweet fennel has about 2.1% of essential oil whereas bitter fennel is more rich (4.7%).

de Vottero et al. (1980) indicated that flowers contained more monoterpenes than fruits and it is the contrary for phenylpropanoids.

A few investigations concerned fennel's improvement. In a paper on fennel cultivation, Desmarets (1978) reported that the classic recurrent selection allows to obtain better producing varieties. Other attempts were realized with the *in vitro* technic method.

Garcia-Rodriguez et al. (1978) working on stem pieces of sweet fennel, observed the formation of calluses capable of giving birth to newly formed buds. These buds produced roots easily and then transformed into normal plants. Analysis realized by Paupardin et al. (1980) revealed that the callus had practically no anethol whereas stem and leaves contained an important amount of this substance. Finally, some of these newly formed plants were more rich in anethol than normal sweet fennel, and in some cases, they were even as rich as the bitter variety. Similar experiments were realized on bitter fennel, first, by Badoc (1982), and later by Du Manoir et al. (1985). The results were the same as those obtained with the sweet variety. At that time, the best clones isolated by Badoc and by Du Manoir et al. were inserted in a recurrent selection program. The association of classic genetic and micropropagation method allowed to create varieties whose yield increased by 50%.

The preceding publications bring to light the differences of essential oil coming from different organs, and from different varieties of different origins. And they have shown means permitting an improvement of fennel.

This brief review shows clearly that chemicals and histological data were not compared. Some research workers considered only the first aspect and others investigated only the second point of view. We decided to realize this comparison by submitting each sample to histologic and chemical analysis.

Material We have worked with two varieties of *Foeniculum vulgare* Miller, *F. vulgare* Miller var. *vulgare* (bitter fennel) and *F. vulgare* Miller var. *dulce* (Miller) Thellung (sweet fennel).

The samples were taken from improved population cultivated near Paris. In the case of the sweet variety, which is annual, sowings were realised in April. In the case of the bitter variety which is perennial, we cut off plants during the second year of growth. For both varieties, samples were taken late June or mid-July. Plants were 0.50 m high for the sweet fennel and 1.40 m for the bitter variety.

Histological methods The samples were fixed with the Regaud and the Meves reactives; this contains osmium tetraoxide which is reduced by the essential oil and produces a brown color.

Then they are included in parrafin and cut into 15 μm thick sections stained with safranin and methylene blue and observed under the photonic microscope.

Analytic methods Material is freezed and crushed into pentane which dissolved essential oil. An internal standard (methyl nonanoate) is added to the extract and this is concentrated with a Kuderna apparatus. Analysis is obtained by gas chromatography; we used a capillary column made of melted silica covered with carbowax 20M. Temperature is programmed from 50°C to 180°C on the basis of 3°C a minute; Helium was the vector gas. Identification of the constituants is obtained by comparison of their retention times with those of known substances.

All histological observations and chemical analysis relating to one experiment were realised with a single plant, at different levels and at the same time, in order to obtain an absolutely correct comparison between data. Usually, samples were collected in late June whereas plants were not yet at the end of their vegetative cycle; at this time, the fruits were not yet ripe. In the case of bitter variety, the fruits exhibit the same degree of ripeness as the sweet fennel only in mid-July. For this reason, harvesting of the bitter fruits was realised two weeks after the one of the sweet fruits.

Results

A. Analytical and histological data. First of all, we will consider for each organ, the structure of the secretory apparatus and the chemical composition of its essential oil.

I. Root

1) Analytical results. The root contains little essential oil: 1.2 mg/g for sweet fennel and 1.6 mg/g for the bitter variety (expressed in fresh material). The root essential oil extracted from sweet fennel contains 81% of dillapiol and 19% of monoterpenic hydrocarbons. The root oil coming from the bitter variety contains 82% of dillapiol, 0.6% of trans-anethol and small amounts of monoterpenic hydrocarbons (Tab. 1 and 2). But a distinct analysis of xylem and cortex revealed differences between these two tissues. We have considered only the sweet variety; the root cortex contains 1.2 mg/g of dillapiol and a small amount of myrcene (Tab. 3). Xylem has less essential oil than cortex and, ahove all,

Tab. 1. Major components of essential oil extracted from various organs of bitter fennel. Amounts of each component are expressed in μg/g of fr. wt. (results got in late June).

Organs	monoterpens				phenylpropanes			
	α-pinene	limonene	myrcene	fenchone	estragol	t-anethol	anisaldehyde	dillapiol
Root	37	51	51	—	—	9	—	1400
Stem	—	50	44	—	—	257	—	—
Petiole	—	21	—	traces	traces	170	9	—
Lamina	100	1512	29	38	317	1864	48	—
First-class inflorescential peduncle	31	316	—	176	38	1034	—	—
Second-class inflorescential peduncle	401	38	—	284	62	1788	—	—
Immature fruits	598	202	617	230	137	3419	—	—

Tab. 2. Major components of essential oil extracted from various organs of sweet fennel. Amounts of each compound are expressed in μg/g of fr. wt. (results got in late June).

Organs	monoterpens			phenylpropanes			
	α-pinene	limonene	myrcene	estragol	t-anethol	anisaldehyde	dillapiol
Root	68	46	103	—	—	—	1002
Stem	181	45	—	—	89	—	—
Petiole	157	167	—	traces	327	traces	—
Lamina	112	686	147	23	690	138	239
First-class inflorescential peduncle	235	235	149	29	943	—	—
Second-class inflorescential peduncle	233	107	144	102	2863	27	—
Immature fruits	178	1433	348	301	7468	—	—

Tab. 3. Comparative amount of essential oil from different underground organs of sweet fennel. The amount of each component is expressed in μg/g of fr. wt.

	monoterpens			phenylpropanes			
	α-pinene	limonene	myrcene	estragol	t-anethol	anisaldehyde	dillapiol
Whole root	68	46	103	—	—	—	1002
Xylem	204	—	—	—	—	—	129
Cortex	—	72	111	—	—	—	1252
Collar	—	36	56	—	—	—	669
Item bottom	59	34	—	—	34	—	177

the two essential oils exhibit very different compositions. Cortex oil is ten times more concentrated in dillapiol than xylem, but it contains no pinene which has been detected in xylem. Finally, xylem is deprived of limonene and myrcene which are present in cortex; these differences are really remarkable, for, they concern two tissues pertaining to the same organ.

2) Histological characters. The root possesses a great number of secretory canals; more than a hundred were counted on a root section from sweet fennel and about fifty in the case of the bitter one; these canals are present in the cortex as well as in the phloem and the xylem. On a transverse cutting (Fig. 1) of the root, canals located in the cortex show a cavity dimly elliptic; axis are about 70×52 μm for the bitter variety. On longitudinal sections, cortex canals are not long and are in fact, secretory sacks. On the other hand, cortex canals show, on a transversal section, only three to five cells in the bitter fennel and about the same number in the sweet one (Fig. 1). Xylem and phloem canals differ obviously from those contained in the cortex: their diameter is so small that on a transversal section, they can only be seen thanks to Meves fixative, whence osmium tetraoxide is reduced by the essential oil.

II. Stem.

1) Analytical data. Stem has a lot less essential oil than the root: 0.31 mg/g of fresh weight for sweet fennel and 0.36 mg/g in the case of the bitter

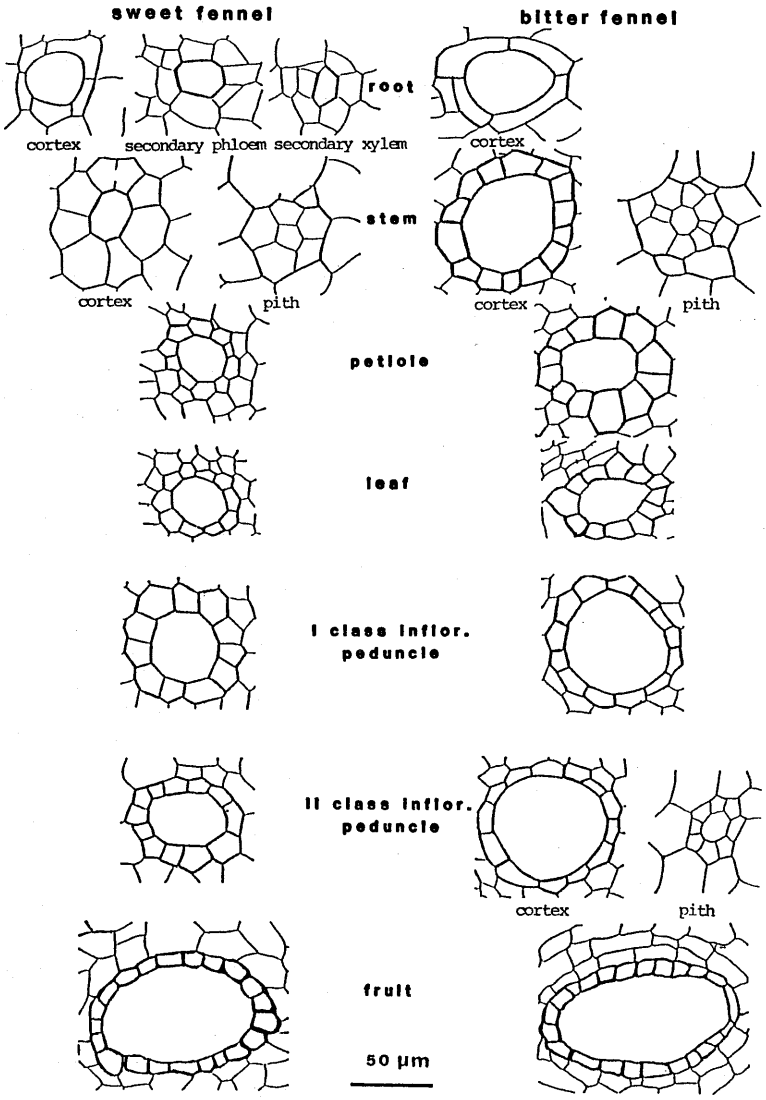

Fig. 1. Transverse sections of the principal kinds of fennel secretory canals.

variety. The composition of this essential oil is considerably different from that of the root. The main component is anethol for the two varieties: the oil of sweet fennel contains 82% of t-anethol and 70% in the case of the bitter one. There is no dillapiol in the stem.

2) Histological characters. On a section practised in the middle part of the plant in either varieties, the total surface occupied by the secretory canals represent one per cent of the surface of the section; however, the cortical canals are more numerous in the sweet fennel (3.5×) than in the bitter fennel, but they are smaller (91 μm–79 μm) for the sweet than for the bitter (106 μm–82 μm) (Fig. 1).

Secretory cells of the cortex canals have thick walls, whereas, in the pith, these secretory cells have thin walls.

III. Leaf.

1) Analytical data. Leaves were taken off from the middle part of the stem; lamina and petiole were analysed separately. For the two varieties, lamina contains more essential oil than petiole. Lamina of sweet fennel has about 2 mg/g of essential oil (expressed with regard to fresh weight). This oil contains 33% of t-anethol, 11% of dillapiol and 1% of estragol. Bitter fennel lamina contains 3.9 mg/g of an essential oil composed of 47% of t-anethol, 8% of estragol and 1.8% of fenchone. In addition, it is possible to detect terpens such as limonene which are not or only slightly present in the petiole (Tab. 1 and 2). It is interesting to point out that the two varieties of fennel differ in the presence of dillapiol in the lamina of the sweet variety. Then, dillapiol is specific of the root in the case of bitter fennel and not for the sweet fennel.

2) Histological characters. In the two varieties, secretory canals of the petiole are similar to those of the stem. Lamina of bitter fennel shows only one secretory canal in front of the main vascular bundle; lamina of sweet fennel contains three canals. However, the canals present more cells in bitter fennel than in sweet fennel; numerous in sweet fennel average diameter is about 58 μm. In bitter fennel, canal section is elliptic; the average diameters are 85×57 μm.

IV. Inflorescence stalks. All the samples were cut off from the primordial umbel and we have considered first- and second-class peduncles separately.

1) Analytical data. The first-class inflorescential peduncle contains about 1.6 mg/g of essential oil (expressed in fresh weight); in the case of sweet

fennel, this essential oil consists mainly of 56% of t-anethol, 16% of estragol and small amounts of monoterpenic hydrocarbons.

The same peduncle of the bitter variety contains similarly 1.6 mg/g of essential oil, but this has a different composition; it contains 65% of t-anethol, 2% of estragol, 11% of fenchone and monoterpenic hydrocarbons.

The second-class peduncle is more rich: 4.5 mg/g in the case of sweet fennel; its essential oil contains 82% of t-anethol and 2.9% of estragol. Second-class peduncle of bitter fennel contains 2.6 mg/g of essential oil; this has 69% of t-anethol, 2.4% of estragol and 6.8% of fenchone.

2) Histological characters. Inflorescential peduncle possesses numerous canals; about thirty can be seen on a transversal section, in the first-class peduncle of sweet fennel and about forty in the bitter variety. Each secretory canal shows twice (Fig. 1) more cells in the bitter fennel than in the sweet one. The cortical canals have a circular section and their diameter is relatively important: (84-89 μm) for the sweet variety and (86-96 μm) for the bitter fennel.

Pith canals are smaller: in addition, secretory cells of cortical canals have thick walls, whereas secretory cells of pith canals show thin walls.

The second-class peduncle have less canals than the first-class ones: 9 in the case of the sweet fennel and 14 for the bitter one. The number of cells by canal is the same in the two varieties; the diameter of the canals is relatively important specially in bitter fennel (64-83 μm for sweet variety and 89-97 μm for the bitter). Secretory cells have thick walls.

V. Fruit. We used unriped fruits being still green. But in the case of bitter fennel, we considered not only green fruits, but also very young fruits which exhibit a yellow coloration.

1) Analytical data. The essence contents of immature fruits are much higher than in the other organs: 9.8 mg/g (expressed in fresh weight) for sweet fennel and 5.2 mg/g for the bitter variety harvested at the same time. Then, the bitter fruits are less mature than the sweet. In order to get valuable comparisons, we have analysed bitter fruits two months and a half after the sweet fruits. In these conditions, the essence content of bitter akenes was about 15 mg/g, consequently much higher than for the sweet fruit at the same stage of development.

The essence extracted from the fruits of sweet fennel (Tab. 2) contains 76% of t-anethol and 3% of estragol, whereas essence from bitter fruits has

Tab. 4. Change of fruit essential oil from bitter fennel during the course of ripening. The amount of each component is expressed in μg/g of fr. wt.

	monoterpens				phenylpropanes			
	α-pinene	limonene	myrcene	fenchone	estragol	t-anethol	anisaldehyde	dillapiol
Very young fruits (yellow coloured)	685	707	116	3120	252	6094	—	—
Immature fruits (green coloured)	103	520	503	4258	393	9213		

only 65% of t-anethol, 5.4% of fenchone and 2.6% of estragol (Tab. 1). No new substance appeared during the ripening, but the quantitative ratios of the different compounds are modified (Tab. 4) : especially the relative quantities of t-anethol, fenchone and estragol increase, whereas the pinene reduces.

2) Histological characters. Fennel's fruit has 6 to 10 secretory canals. The average number is 6.3 for the sweet variety, this number being affected by 7.2% as variation factor. The akene of bitter fennel has an average number of canals equal to 7.8 with a variation factor of 9.2%.

These canals are very large. (Fig. 1) Their transversal sections are elliptic. The average size of the diameters are 128×79 μm for the sweet fennel and 137 ×78 μm in the case of the bitter variety. Walls of the secretory cells are particularly thick. The average number of secretory cells counted on a transversal section is about 15 for a canal in the sweet fennel and 19 in the case of the bitter variety. (To be continued)

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2種類のウイキョウ bitter fennel (*Foeniculum vulgare* Miller var. *vulgare*) と sweet fennel (*F. vulgare* Miller var. *dulce* (Miller) Thellung) を材料として，根，茎，葉，花柄，果実各器官における精油含量・組成の分析，及び，組織学的観察を行った。

精油含量・組成は器官により大きく異なり，茎，葉柄では含量が低かったが，果実では非常に高かった。組成は，根では dillapiol が多く検出されたのに対し，地上部では

それは sweet fennel の葉身でのみ検出されただけで，bitter fennel では検出されなかった。又，t-anethol は地上部において主要な精油成分であった。一方，組織学的観察から，器官によって分泌管の構造や大きさ，数が異なっていた。

○高等植物分布資料（129）Materials for the distribution of vascular plants in Japan (129)

○マヤラン *Cymbidium nipponicum* (Franch. et Savat.) Makino　1989年7月18日，東京都文京区の東京大学構内のスダジイ樹下で，つぼみの状態の花茎を発見した。他に付近には花茎は見当たらず，枯れた茎も見当たらなかったので，本年がこの個体の最初の開花年に当たるものと推定された。その後観察を続けたところ，7月24日になって開花を見，本種であることを確認した（図1)。つぼみの時点では，花茎にはつぼみを含めて葉緑素によると思われるような着色は認められず，全体に赤褐色と紫褐色を帯びていたが，白地に紫紅色の条線の入った花を咲かせた後，子房部位を中心に淡緑色の着色が認められるようになり，子房が肥大するとともに花茎もアントシアン性の紫色の着色を失い緑色を呈するようになっている。なお，一花は人工授粉を行なって結実を見たが，他の放置した花も結実を見た。以上は塚谷の観察によることが多い。

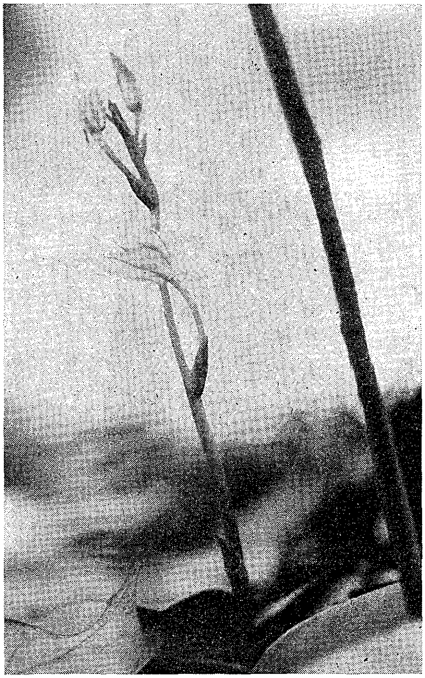
図 1.　マヤラン *Cymbidium nipponicum.*

本種は腐生性のため，個体数が少なく，また地上部が花茎のみであるため発見されることも稀である。分布は関東南部以西とされているが，そうした事情から，東京の市街地の中での自生例は稀なことと思われる。花茎の標本は東京大学総合研究資料館におさめた。

（東京大学 遺伝子実験施設 塚谷裕一
Hirokazu TSUKAYA・東京都文京区[redacted] 津山 尚 Takasi TUYAMA)